GROWTH AND MORPHOGENESIS IN CRUSTACEAN LARVAE

JOHN A. FREEMAN

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Crustaceans grow in a step-wise manner that is a function of the tissue growth during the moult cycle combined with stretch at ecdysis, moult increment, and moult cycle duration. In larvae, growth of the epidermis is also involved in the morphogenesis of integumental structures. To understand the mechanism and regulation of growth and integumental morphogenesis, tissue growth was studied in *Palaemonetes* larvae and *Artemia* metanauplii. Increase in carapace length in *Palaemonetes* larvae is proportional to the growth of the underlying epidermis. Moreover, growth in the epidermis is highly correlated with that of the muscle. Both tissues show the greatest amount of growth during the first third of the moult cycle. Tissue growth and carapace size were not affected by rearing temperature or exposure to the moulting hormone (20-hydroxyecdysone), factors that strongly affect moult cycle duration. Another factor, feeding regime, markedly affected tissue and carapace growth. The most critical period for food intake is the first third of the moult cycle. It is uncertain, however, whether nutritional state controls tissue growth directly or through other physiological processes. At ecdysis, hydrostatic pressure, under the control of the neurosecretory center, expands the new integument to a point equal to the amount of new tissue generated during the previous moult cycle. Although eyestalkless larvae grew more in carapace length than intact larvae, there is no difference in the amount of growth of the epidermis between the two groups. Uptake of water at ecdysis, then, serves to expand the new cuticle but, under normal conditions, does not affect tissue growth. Studies on segment morphogenesis in *Artemia* reveal that patterned cell replication is involved in growth and shape of the thoracic integument and, as a morphogenetic force, leads to regional differences in cell density that are integral to formation of the arthrodial membrane and the thoracopod limb bud. The cell replication pattern may, in fact, be an initial step in cell differentiation. Thus, epidermal growth and morphogenesis in crustacean larvae is a function of both the nutritional state of the organism and cellular events that control development. □ *Crustacea, larvae, growth, morphogenesis.*

John A. Freeman, Department of Biological Sciences, University of South Alabama, Mobile, Alabama 36688, USA; 6 *July, 1990.*

The presence of a restrictive exoskeleton in larval and adult crustaceans limits expansion of the integument to periods of ecdysis. The corresponding growth curve appears to rise in a step wise manner (Hartnoll, 1982; Botsford, 1985). The growth curve is a function of the time between ecdyses, or moult cycle duration (MCD), and the amount of linear growth that takes place at ecdysis, or moult increment (MI). These factors have been recently reviewed in depth by Hartnoll (1982) and Gore (1985). The growth curve is generally species-specific and defines the relationship between larval size and duration of the larval period. Variation in the curve can result from sexual maturation, environmental factors (chiefly temperature), nutritional stress, and parasitism (Hartnoll, 1982).

Crustacean larvae undergo a series of larval moult cycles, hereinafter referred to as instars, before metamorphosis to the juvenile form. For each species, the number of instars may be constant or may vary under certain environmental conditions (Broad, 1957b; Knowlton, 1974). Some degree of morphogenetic change is observed at each instar, and the schedule of the changes is in most cases so invariant that the morphogenetic state defines that particular instar (Broad, 1957a; Williamson, 1982). Development may be gradual (direct) with slight change at each ecdysis (e.g. *Homarus*, *Artemia*) or there may be one or more major changes in body form (indirect), as exhibited by barnacles and penaeid shrimp (Walley, 1969; Williamson, 1982). For each larval instar, the organism grows in size and changes form. Although these changes are manifested at ecdysis, the tissue changes occur continuously throughout the moult cycle. The constancy of growth and morphogenesis in the larval instars would suggest that both processes are interrelated and may be regulated by a common mechanism. However, this mechanism has yet to be elucidated.

In our studies of the control of growth and morphogenesis in crustacean larvae, we have examined the process of growth in larvae of the caridean shrimp, *Palaemonetes pugio*, and growth and morphogenesis in larvae of the brine shrimp, *Artemia*. Since linear dimension is a function of the cuticle and the epidermis, growth in this tissue was used as a tool to differentiate between the factors that regulate moulting and MCD and those that control MI (Fig. 1A,B). The larval epidermis is a two dimensional monolayer of cells whose primary role is to carry out the cyclical degradation of the old cuticle and synthesis of the new cuticle. The cells actively participate in this function throughout most of the moult cycle with only a brief resting (intermoult) period (Freeman and Costlow, 1980; McConaugha, 1985; Christiansen, 1988). In addition to its role in cuticulogenesis, the epidermal cell replicates to increase the area of the monolayer and differentiates to form specific integumental structures.

MOULT CYCLE DURATION

A primary component of growth is the moult cycle duration (MCD). The length of the moult cycle is temperature dependent, relatively constant, and species-specific in the larval phases, while the juvenile and adult moult cycles increase in length with age. Although the regulation of the length of each stage of the moult cycle remains unclear, it is widely accepted that the endocrine system controls the onset of premoult and ecdysis. During most of the intermoult period, the onset of moulting is blocked by moult-inhibiting hormone (MIH), a peptide secreted by the eyestalk neurosecretory centers (Chang, 1985; Skinner, 1985). Cessation or reduction in production of MIH are presumed to initiate the onset of proecdysis. This has been shown for adults (Skinner, 1985) but has not been consistently observed in larvae. Eyestalk removal did not stimulate moulting in crab larvae (Costlow, 1966a,b) and shrimp larvae (Little, 1969). A more frequent observation schedule revealed that eyestalk removal was effective in shortening the moult cycle (Freeman and Costlow, 1980). The findings from these studies clearly show that the larvae are traversing the moult cycle at a rapid rate that can be only slightly accelerated.

Promotion of the onset of premoult is controlled by ecdysone and 20-hydroxyecdysone (20HE)(Skinner, 1985). This regulatory step was demonstrated for larvae in barnacles (Freeman and Costlow, 1983a), and crabs (McConaugha and Costlow, 1981; Freeman and Costlow, 1984) when larvae exposed to 20HE entered premoult prematurely. Recently, ecdysteroid levels have been determined in lobster larvae (Chang and Bruce, 1981) and in crab zoeas (Spindler and Anger, 1986; Anger and Spindler, 1987). In both studies the hormonal titres resembled those of adults in that the concentration declined following postmoult, remained at a basal level for the short period of intermoult and then peaked during mid premoult. Moreover, by comparison to hormone profiles in adult crustaceans, the premoult rise appears to begin earlier than stage D_0 in some instars. Chang and Bruce (1980) also showed that the profile was similar during multiple, shortened moult cycles in eyestalkless lobster juveniles. The titre increased at the onset of premoult, which began earlier, suggesting that the titre can undergo normal cycles even in the absence of MIH. Thus, the regulation of moulting hormone levels may be under the control of more factors than just MIH.

Temperature has been shown to be a strong regulator of moult cycle duration in crustacean larvae. There is generally an inverse relationship between temperature and MCD (Knowlton, 1974; Hartnoll, 1982). At the lower temperature range the MCD will be several times longer than that seen at the optimal temperature. In some cases, the larvae will never moult. At the higher temperature range, the MCD will reach a point where it cannot be further accelerated.

To find if the amount of growth during an instar was dependent on the MCD, temperature was used to control the MCD in instar II *Palaemonetes* larvae. The MCD was inversely proportional to a temperature range of 15–30°C with larvae at the lowest temperature (15°C) demonstrating moult cycles of almost seven days compared to a two day MCD for those at 30°C (Freeman, 1990b). The MI, however, was greatest at 20 and 25°C with the least amount of growth at 30°C. The results show that growth of the carapace then was dependent on temperature but it was independent of the MCD. Moreover, since all moult cycle stages were lengthened or shortened proportionately by these treatments, there does not appear to be one phase of the moult cycle that must be of a certain minimum duration in order for growth to occur.

Moulting hormones accelerate the onset of the premoult period and thereby shorten the intermoult period in larvae of all crustaceans examined (Skinner, 1985; Christiansen, 1988).

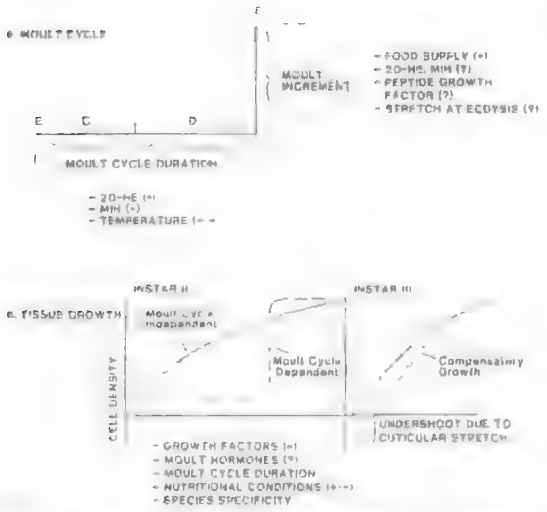

FIG. 1. Model of the growth process in crustacean larvae. A, Factors that control growth during one moult cycle, shown as part of a growth curve. The moult cycle is the period between ecdyses (E) and is divided into postmoult and intermoult (C) and premoult (D) after Drach (1944). Factors that control moult cycle duration include 20-hydroxyecdysone (20HE), moult-inhibiting hormone (MIH), and temperature. The amount of growth at ecdysis, or moult increment, is affected by food supply, stretch at ecdysis, and possibly moulting hormones and growth factors. B, Growth of tissues during the moult cycle. Growth could be moult cycle stage dependent if it occurs primarily during one stage of the moult cycle, or moult cycle stage independent if it occurs throughout the moult cycle. Here for example, the growth occurs during early premoult. When growth of the epidermis is seen as change in cell density, the density will increase during the molt cycle and then return to a basal level at ecdysis, as shown here for the change at ecdysis to instar III. After ecdysis, the density will again increase. If a greater than normal amount of stretch occurs, the density value will fall below the basal level (undershoot). The tissue may (compensatory growth) or may not be able to obtain the same density. Tissue growth is influenced by nutritional conditions, genetic factors (species specificity), and, possibly, growth factors, moult controlling hormones and moult cycle duration.

Treatment with 20HE was used as a method to shorten the MCD in order to further examine the relationship of MCD and growth and to explore the role of moulting hormones in the growth process (Freeman, 1990b). Exposure of instar II *Palaemonetes* larvae to 20HE shortened the postmoult and intermoult periods by 33% but did not noticeably affect the duration of premoult. There was no difference in carapace length of the control and hormone-exposed larvae. These findings support the contention that growth in crustacean larvae is not dependent on the MCD. The results also suggest that moulting hormone does not have any direct influence on growth of the larvae. The endocrine system, then, controls the moult cycle and the rate at which intermoult tissue growth is realised at ecdysis. However, the moulting hormone may possibly play a permissive role in growth by establishing conditions conducive to growth of the epidermal cells, e.g. cell division in late premoult.

The conditions tested (2 day MCD, temperature control; 36 hr intermoult stage C, 20HE control) did not permit a continuous attenuation of the MCD below a certain point. At some point in the abbreviation process, the larva would probably lose the ability to accumulate food reserves. At that point the synchrony of the complex cellular events would be disrupted, which would lead to decreased growth. Since one day of feeding is required to successfully moult (Freeman, 1990b), this may be the critical duration although even lesser periods may be possible at higher temperatures.

An obvious candidate for regulation of MCD is the quality and quantity of the food. Many studies have examined the role of nutrition in crustacean growth, metamorphosis and survival (Broad, 1957b; Knowlton, 1974; Hartnoll, 1982; Gore, 1985; McConaugha, 1985). As expected, low rations of food limit growth. This result is confirmed during studies on the effect of feeding regime in shrimp larvae (Freeman, 1990a). Several studies have clearly shown that normal MCD and morphogenesis is controlled by the nutritional condition of the larva (Broad, 1957b; Anger *et al.*, 1981; McConaugha, 1982; Anger, 1984; West and Costlow, 1988).

MOULT INCREMENT

Although increase in linear dimension is observed at ecdysis, the growth process occurs throughout the moult cycle (Frank *et al.*, 1975; Sulkin *et al.*, 1975; Anger *et al.*, 1989). These data, obtained primarily from biochemical analyses, contain little information on the actual growth of the tissues. In this section I consider factors that: 1) play a role in the moult increment (MI) in the organism and influence growth of the tissues during the moult cycle, and 2) control expansion at ecdysis.

Any consideration of a growth mechanism must begin at the level of the gene. The disparate sizes are obviously a function of the genetic

mechanism that controls the MI and, to a lesser degree, the MCD. For each species, there appears to be a process that establishes a basal MI under normal conditions (Rice, 1968; Hartnoll, 1982; Gore, 1985; Freeman, 1990b). External factors may act on this process directly or may control the rate at which the system operates. Future efforts aimed at understanding the genetic control of growth will greatly contribute to general knowledge of growth in crustaceans and other organisms.

Built into the growth controlling system in larvae is the ability to somehow recognise their size or 'growth state' and to grow accordingly. A larva which is smaller than the mean size for that species/instar/cohort may grow more than larger larvae of similar age, and vice versa. This has been shown for *Palaemon* (Hartnoll and Dalley, 1981), barnacle (West and Costlow, 1987), and *Palaemonetes* (Freeman, 1990b). The manner in which this system functions is not understood.

Temperature appears to affect growth by controlling metabolism. There is an optimum temperature at which food conversion and anabolic processes take place and, as mentioned above, an optimum temperature for progression through the moult cycle. Growth probably occurs most efficiently at a temperature where the optima coincide. For larvae of *Palaemonetes pugio* this optimum is 25°C.

Growth controlling hormones may include those that regulate moulting (mentioned above) and the hormone(s) that regulate hydromineral content. Eyestalk removal in crustaceans has been shown to significantly decrease the intermoult period under conditions where the moult cycle progression is carefully monitored, indicating the presence of MIH in larvae. In addition to regulating the MCD, MIH could actually affect the growth behaviour of the cells, although no evidence of such a role for MIH has been presented. Growth does not appear to be affected by MCD and the neurosecretory system is incomplete in early larval instars. Thus, it is unlikely that MIH participates in growth regulation other than the control of the MCD.

In addition to moult cycle acceleration, eyestalk removal results in a postmoult size that is larger than the comparable value for intact animals. This has also been shown to be true for eyestalkless larvae of *Rhithropanopeus* (Kalber and Costlow, 1966; Freeman *et al.*, 1983), *Homarus* (Charmantier *et al.*, 1984; Snyder and Chang, 1986), and *Palaemonetes* (Okazaki *et al.*, 1989; Okazaki, pers. comm.). The increased size is thought to be due to enhanced uptake of water as a result of loss of the hormone that controls hydromineral balance (Mantel and Farmer, 1985). The higher than normal hydrostatic pressure stretches the new, soft integument beyond the limits established by tissue growth during the previous instar. Thus, the stretch before hardening of the cuticle supercedes the growth potential.

The mechanism of tissue growth under the conditions of supranormal haemolymph hydrostatic pressure is beginning to be studied. Freeman *et al.* (1983) found that the density of the epidermal cells of juvenile *Rhithropanopeus harrisii* that had undergone four or five moult cycles following eyestalk removal during the larval period was greater than that of intact animals although the carapace was much larger. These results indicated that the epidermal tissues of eyestalkless larvae somehow compensated for increased cuticle area after ecdysis by increasing cell growth and replication. Conversely, Okazaki *et al.* (1989) found that the increased stretch in eyestalkless larval and adult shrimp was not accompanied by compensatory growth of the cells. Instead, the density remained lower than that of the intact animals. Comparable studies with different species are needed to find 1) the most common growth process for crustaceans, and 2) if the response to stretch differs between stages of the life cycle within a single species.

Not unexpectedly, nutritional state is a strong regulator of growth. Many studies have shown that food deprivation or differences in food quality has a marked effect on growth (Hartnoll, 1982; McConaugha, 1985; Anger, this volume). Recently, the feeding regime was used to examine the growth processes in *Palaemonetes* larvae (Freeman, 1990a). Feeding during the first two thirds of the moult cycle did not markedly affect the MI, indicating that sufficient food was stored during the intermoult and early premoult periods. Feeding only on the first or second days of the instar, however, resulted in levels of growth that were intermediate between starved and continuously fed controls with more growth observed in those larvae fed on day 1 of the instar. These findings indicate that feeding during the first third of the moult cycle is critical for successful growth. This period corresponds to the 'point of reserve saturation' (Anger and Dawirs, 1981) or the threshold for growth and development (West and Costlow, 1988), while feeding on day 2 may be sufficient to reach the

'D_0 threshold' and moult, but with only slight growth (Anger, 1987). The first third of the moult cycle is clearly a period in which food assimilation is somehow monitored and translated into actual tissue growth or stored for further growth and morphogenesis. Why this phase should be so essential is not obvious. Several studies have shown that tissue growth occurs at this time (Frank *et al.*, 1975; Sulkin *et al.*, 1975; West and Costlow, 1987; Anger *et al.*, 1989; Harms *et al.*, 1990). Feeding may become more active because apolysis and premoult changes have not yet begun. Interestingly, in the *Palaemonetes* feeding study, some growth occurred in starved larvae that survived instar II. This growth may have been fueled by food reserves accumulated during the previous instar or may have resulted from tissue catabolism.

CELLULAR BASIS OF INTEGUMENTAL GROWTH IN CRUSTACEAN LARVAE

To comprehend growth of the cuticle, it is necessary to understand how the process occurs in the underlying epidermis. This is best accomplished by considering the cell cycle with respect to the moult cycle. Following the beginning of premoult, the epidermis increases in cell number by mitosis (Tchernigovtzeff, 1965; Halcrow, 1978). In some larvae mitosis may occur in periods of the moult cycle other than premoult (Le Roux, 1978; Freeman, 1986). In either case, cell replication slightly increases the area of the epidermis. Cell replication and growth may occur to the extent that, in transverse section, the epidermis appears to be folded (Skinner, 1962; Freeman and Costlow, 1980). At ecdysis, the uptake of water (DeFur *et al.*, 1985) expands the new integument to a point that will equal its new size (Freeman, 1990b).

Little information exists on the control of the cellular processes by external and internal factors. To establish a conceptual framework, a model of the system relating tissue growth and the moult cycle is shown in Fig. 1B. The instar II larva of *Palaemonetes pugio* is well-suited for this model since the epidermal cells of dorsal carapace exist as a monolayer which is spatially restricted so that its growth can be compared to change in the carapace dimensions (Fig. 2).

The epidermal cell density is found to increase only during the first day of the moult cycle in normal larvae (Freeman, 1990b) (Fig. 3). At the end of this period the predicted growth can be determined from the cell density. This is also the period when maximal growth of the muscle occurs (Schaff and Freeman, unpubl.). Preliminary findings show that the increase in cell density is a result of enlargement of certain cells within the epithelium. The cells that enlarge appear to be cells that have divided during premoult of the previous instar. Their expansion slightly compresses other cells in the monolayer, and results in the enhanced cell density.

Factors that affect MI do so through the growth of the epidermis. Growth of the epidermis in *Palaemonetes* is greatest at the optimum temperature of 25°C (Freeman, 1990b). Larvae exposed to moulting hormone show no differences in the epidermal growth, as was the case for MI. The strong effect of nutrition on MI is reflected in the cellular growth. An example of this relationship is shown in Fig. 4. Starved larvae underwent little growth in the epidermis while larvae fed on day 1 demonstrated cell growth that was intermediate between fed and starved larvae. A direct correlation was observed between the increase in density by day two of the instar and the MI (Freeman, 1990b)

The tissue, then, is able to respond to the nutritional state (or food storage) very rapidly by growth of some cells leading to increased density. Since the density does not increase as much in starved larvae, the process of enlargement (G1 growth ?) may be controlled by sufficient uptake of food or uptake of certain compounds. The other growth phase is the premoult period when cell division takes place. Little is known of how this phase is controlled by food uptake. One possiblity is that the cell cycle is controlled by nutritional state such that a cell will make a decision to continue through S, G2 and mitosis as a result of some signal propagated after feeding. Alternatively, a certain number of cells are stimulated to cycle during the feeding-sensitive period but that other factors, generated during the remainder of the moult cycle, would control their division. Several control points in the eukaryotic cell cycle have been shown to exist. A major point is the G1-S (or G1a-G1b) transition (Pardee, 1989). In the shrimp epidermis this may be seen as the enlargement of a few cells. Another control point is the G2-M transition (Murray and Kirschner, 1989). Since mitosis is most often seen in premoult, this decision may be one that is independent of the transition to S phase. These intriguing hypotheses must be empirically tested to determine how the epidermal cells are regulated by food intake and nutrition.

From our preliminary studies it is clear that only

FIG. 2. Nuclei of the dorsal epidermis of live instar II *Palaemonetes pugio*. The nuclei are vitally stained with a nuclear fluorochrome bisbenzimide (Hoechst, 33342).Fluorescence image. Bar = 50 µm.

certain cells, or a certain number of cells replicate during the moult cycle. This has been found to be true for *Palaemonetes* larvae where the positions of dividing and enlarging cells appear to be constant. Moreover, positional information may be used to determine if a cell is to divide or remain in the non-cycling compartment. Within the monolayer some cells will leave the cycling population as growth and morphogenesis proceed. How nutritional state effects the regulation of these events is not understood.

GROWTH AND MORPHOGENESIS

The crustacean epidermis represents an unusual example of cell differentiation in that the function of cuticulogenesis is fully developed at hatching but the cells must also form the morphologically different regions of the integument. In this process, they will further differentiate to a more specialised type of epidermal cell. Among the specialised cells are setal cells, tendinal cells, transport cells, and, in developing nauplii of some species, the neuroblast cells (Freeman, 1989a). Each of these cell types begins as a cell in the general larval epithelium. As the tissue grows and develops, the cell changes and differentiates. Following differentiation, the cell may not grow further or, if it is part of a growing region of the integument, it may continue to divide as the region grows in proportion to other areas of the integument. For many species of crustaceans another dramatic change will occur when the larva undergoes metamorphosis. In some instances, this change involves only a transformation from a caridoid to a cancroid form. In others, an extreme change takes place, e.g. the barnacle cyprid (Walley, 1969). While a discussion of all of the cellular and regulatory processes is beyond the scope of this review, the process of cell growth is essential to the generation and maintenance of form and shape of the integument and will be discussed here.

Crustacean larvae hatch from the eggs at different stages of larval development (Williamson, 1982; Gore, 1985). Some may be advanced and undergo little postembryonic development (e.g. *Homarus*), while others may hatch at the nauplius, the least developed larval form of the Crustacea. At each ecdysis some amount of growth occurs and developmental complexity increases. For the nauplius, this is seen as the process of segmentation in the thorax and abdomen, while in the zoea it involves development of limbs in segments that are already formed.

Morphogenesis in decapod crustaceans, the group for which most studies have been accomplished, includes continued development of the

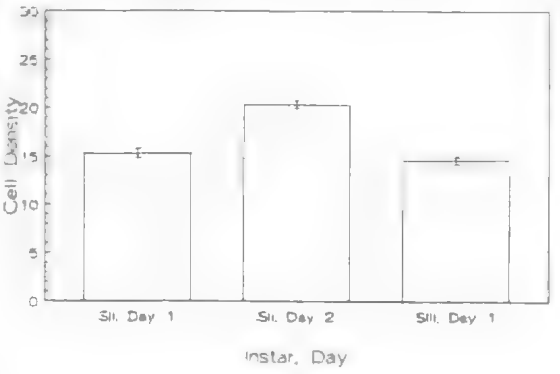

FIG. 3. Change in density of the epidermal cells in the dorsal carapace of live *Palaemonetes pugio* during the second instar. The density increased by approximately five cells during the first day of the instar and returned to the basal level at ecdysis to instar III.

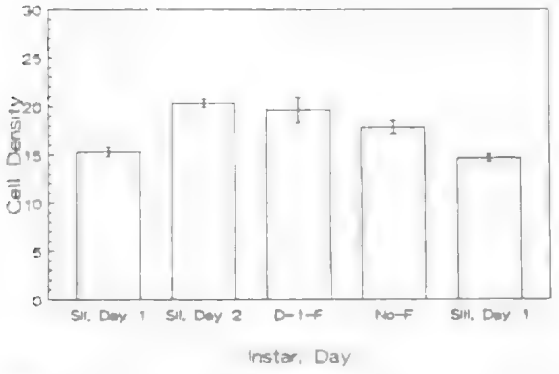

FIG. 4. Change in cell density of the epidermis under different feeding conditions that are known to affect the molt increment (Freeman, 1990a). The density at the beginning of the instar was similar for all groups (SII, Day 1). Fed control larvae underwent the normal increase in density by the end of day 2 (SII, Day 2) while larvae fed only on day 1 (D-1-F) or starved (No-F) showed less growth of the epidermis during the same period. All larvae that moulted to instar III demonstrated the same density early on day 1 (SIII, Day 1), indicating that the larvae that were fed only on day 1 or starved grew less during the instar.

abdominal segments and thoracic legs (Williamson, 1982). The changes are moderate and expand on an already segmented state in which most of the cell types and tissues has been formed. A major transformation ensues at metamorphosis in crabs when the integument changes from a laterally to a dorso-ventrally compressed form.

Segment formation in the nauplius of *Artemia* begins when the pattern of cell replication changes from one that maintains a longitudinal file of epidermal cells to that of a transverse file (Freeman, 1986, 1989a,b). The first transverse file is positioned ventro-laterally in the middle of the presumptive first thoracic segment (Fig. 5). Segmentation proceeds in a anterior-posterior gradient beginning at segment 1 (Weisz, 1946, 1947; Anderson, 1967; Benesch, 1969; Freeman, 1989a,b). The maxillae (cephalon) develop at about the same rate as the first two segments. The first major segregation of integumental regions is the formation of the arthrodial membrane (AM) and the thoracopod bud. Cells of the limb bud continue to divide transversely at a faster rate than the AM cells until a well defined difference in the cell density is established early in instar II (metanauplius 1). This difference in the spatial arrangement of the cells is essential for the evagination process (Freeman, 1989b,c). Within two hours of achieving the differential cell density the AM cells change shape and undergo apolysis earlier than the posterior regions. As a result of the combination of these events the AM region invaginates as the thoracopod bud region evaginates and segment one is defined.

Formation of the thoracopod continues over the next several instars. Throughout this period the spatial and temporal manner of the cell replication is important for 1) outward expansion of the bud, 2) growth of the exopodites, endopodites, and epipodites, and 3) placement of cells for differentiation (Freeman, unpubl.). Special cell types differentiate during this period. The major tendinal cell forms at the end of instar II (Fig. 6) while smaller tendinal cells differentiate in concert with the segmental muscles during instars IV, V, and VI (Freeman, 1989a). The neural precursors leave the epithelium during

FIG. 5. The pattern of epidermal cell nuclei in the ventral epidermis of fixed instar II *Artemia* larvae. Nuclei are stained as described in Figure 2. Anterior is to the top. A longitudinal file is indicated by single arrowhead. The first transverse file of the first segment is indicated by paired arrowheads. (M= midline; Seg 1 = segment 1). Bar = 50 μm.

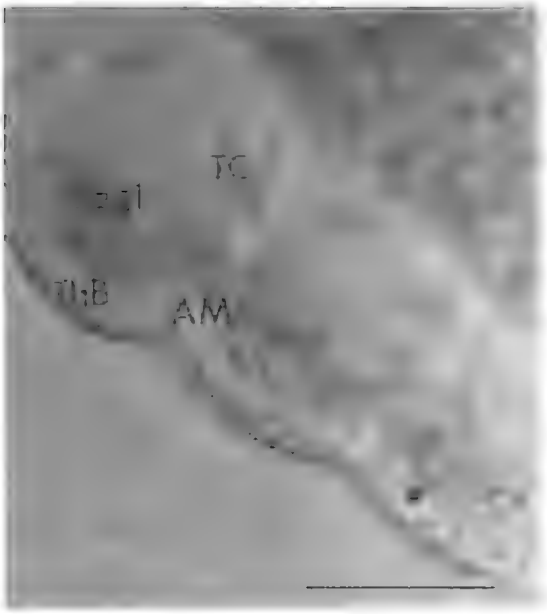

FIG. 6. The segmenting region of the presumptive thorax of live instar II *Artemia* larvae. The thoracopod limb bud (ThB) and the arthrodial membrane region (AM) of segment 1 (Seg1) are shown. The tendinal cell (TC) supports the invaginated AM region. Bar = 50 µm.

instar III or IV and their position within the epidermis may be identified as early as instar II. Finally, the setal cells differentiate during instar VI as the thoracopod is completed. Thus, growth in crustacean larvae leads not only to increased size at ecdysis, but also to segmental structures.

Although numerous papers have described the development of the larval appendages, none have investigated the cellular basis of this development. We must presume that the changes are, in general, similar to the kinds of morphogenetic changes observed in the thoracopod of brine shrimp. As a comparison, the AM region would correspond to the joints of the limbs and the tendinal and setal cells would develop in conjunction with the muscles and integumental structures, respectively. The control of this process is not understood at this time. The events may be preprogrammed within the developing epidermis, or, alternatively, the cells may be responding to extrinsic elements. Although the pattern of segmental development is relatively constant, there is some plasticity within the epidermis. This is demonstrated by the limb regeneration process in which non-limb epidermal cells can generate the entire limb within two moult cycles (Adiyodi, 1972).

Cellular activities other than growth and differentiation are involved in larval development and metamorphosis. Programmed cell death occurs in the antennae of barnacle cyprids following attachment and in the spines of crab zoeas during metamorphosis to the megalopa stage (Walley, 1969; Freeman and Costlow, 1983a,b). This cellular state appears to be developed during the larval period and is activated by hormonal mechanisms. Exactly how growth sets the stage for these events is unknown.

SUMMARY

The integument of the crustacean larva grows and assumes form by a pattern of epidermal cell replication and differentiation that is regulated in a spatial and temporal manner. Growth is independent of the moult cycle duration and moult-controlling hormones. Nutritional state is a strong determinant of cellular growth. The cells grow to a point that determines the growth potential that is realised at ecdysis when the new cuticle expands. Segmentation and limb formation result from the spatial pattern of cell replication within the epidermis. Future endeavors will explore the basis of the cellular events involved in integumental growth and development and the regulatory mechanisms that are involved in these processes.

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